

The Gametophytes and Young Sporophytes of *Elaphoglossum decursivum* (Dryopteridaceae) in Costa Rica

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ABSTRACT.—Most studies on ferns focus on the adult sporophytes, overlooking the gametophyte as a potential source of biological information. Herein, field-collected gametophytes and young sporophytes of *Elaphoglossum decursivum* are described with the aims of providing a more complete understanding on the morphology of this species and additional morphological characteristics for its sectional classification. Gametophytes share the general morphology of most other *Elaphoglossum* species, but one unusual observation was the presence of minute scales (proscas) on the thallus. Young leaves differ from the glabrous adult leaves by having papillate glandular hairs. These are the first observations of hairs in the glabrous sect. *Elaphoglossum*. Our study shows that the gametophytes and young sporophytes of *E. decursivum* have indument characters that are not present in the adult sporophytes, and studying these life stages could potentially be useful in a phylogenetic context.

KEY WORDS.—Morphology, indument, infrageneric classification

Elaphoglossum Schott ex J. Sm. is composed of over 600 species that are morphologically similar and hence difficult to treat taxonomically (Mickel and Atehortúa, 1980; Vasco *et al.*, 2013). To provide a useful framework for future monographic treatments, Mickel and Atehortúa (1980) proposed an infrageneric classification based on morphological characters of the sporophytes and spores, including such characters as rhizome habit, phyllopodia, blade scales, presence versus absence of hydathodes, and perispore morphology. Later, Rouhan *et al.* (2004) and Skog *et al.* (2004) conducted molecular phylogenetic studies using cpDNA on subsets of the genus (123 and 48 species, respectively). Their studies recovered six major clades, most of which were recognized at the rank of section (i.e., sects. *Amygdalifolia*, *Elaphoglossum*, *Lepidoglossa*, *Squamipedia* and *Subulata*), or at the rank of subsection (i.e., *Pachyglossa*, *Platyglossa*, *Polytrichia* and *Setosa*). They recovered *E. amygdalifolium* (Mett.) Christ, the sole species of sect. *Amygdalifolia*, as sister to all other species of *Elaphoglossum*. Recently, a new section of the genus (*E.* sect. *Wrightiana*) was erected to accommodate the early diverging Cuban endemic *E. wrightii* (Mett. ex D. C. Eaton) T. Moore (Lóriga *et al.*, 2014). These molecular

phylogenies largely agree with Mickel and Atehortúa's (1980) classification system and have been useful in resolving major clades within the genus. However, some of these clades are still lacking obvious, non-molecular synapomorphies, for example, the glabrous *Pachyglossa* and *Platyglossa* clades of sect. *Elaphoglossum*.

One potential source of morphological characters that is often overlooked is the gametophytic stage. Comparative studies have found that fern gametophytes can have distinctive morphological and developmental characters useful in distinguishing genera and higher taxa (Atkinson and Stokey, 1964; Farrar *et al.*, 2008; Gabriel y Galán and Prada, 2012; Johnson *et al.*, 2012; Nayar and Kaur, 1971). Gametophyte morphology, however, has yet to be proven useful at the infrageneric level, in part due to the paucity of data. *Elaphoglossum* is no exception. To date, the gametophytes of only about 30 of the 600 *Elaphoglossum* species have been described (Atkinson and Stokey, 1964; Chiou *et al.*, 1998; Lagomarsino *et al.*, 2012; Momose, 1967; Nayar and Kaur, 1971; Perez-Garcia and Jaramillo, 1990; Sánchez-Montiel *et al.*, 2008; Stokey and Atkinson, 1957). Moreover, apart from the Lagomarsino *et al.* (2012) study of *E. amygdalifolium*, none of the other studies discussed *Elaphoglossum* gametophyte morphology in a cladistic framework.

In general, the mature gametophytes of most *Elaphoglossum* species are elongate-cordate, with nearly parallel sides, crisped wings, and shallowly notched apices (Chiou *et al.*, 1998; Nayar and Kaur, 1971; Stokey and Atkinson, 1957). Many of the examined species also show lateral branching in the older gametophytes (Chiou *et al.*, 1998; Nayar and Kaur, 1971; Stokey and Atkinson, 1957). Rhizoids are typically borne on the margins, both sides of the midrib, and on the wings (Nayar and Kaur, 1971; Stokey and Atkinson, 1957), but they are usually most abundant on the ventral side of the midrib (Stokey and Atkinson, 1957). In addition, unicellular glandular hairs are found on the margins and on both surfaces of the thallus (Chiou *et al.*, 1998; Nayar and Kaur, 1971; Stokey and Atkinson, 1957). These hairs contain chloroplasts when young and are often covered by a whitish waxy cap (Chiou *et al.*, 1998; Stokey and Atkinson, 1957). A few notable exceptions to this general gametophyte form have been described. Stokey and Atkinson (1957) found the gametophytes of two species from sect. *Squamipedia* to be hairless throughout development. This observation was recently confirmed by Vasco *et al.* (2013), suggesting that the absence of papillate hairs might be a synapomorphy for sect. *Squamipedia*. *Elaphoglossum amygdalifolium* also differs from the general form of the genus by lacking both marginal rhizoids on the thallus and waxy caps on the hairs. By these characters, it resembles the sister genus *Mickelia* and other bolbitidoid outgroups (Lagomarsino *et al.*, 2012).

Elaphoglossum decursivum Mickel occurs in the wet montane forests of Mexico, Belize, Honduras, Costa Rica and Panama, from 450–850 m (Mickel and Smith, 2004). It is one of the most abundant low-trunk fern epiphytes at Las Cruces Biological Station in Costa Rica, and is most commonly found growing on the trunks of the tree fern *Alsophila firma* (Baker) D. S. Conant (Moran *et al.*, 2003). *Elaphoglossum decursivum* was not included in the

molecular phylogenetic study of Rouhan *et al.* (2004); however, it appears to belong to sect. *Elaphoglossum* of Mickel and Atehortúa (1980) based on its nearly glabrous leaves and presence of phyllopodia. Section *Elaphoglossum* consists of two subsections: subsects. *Pachyglossa* and *Platyglossa*, *sensu* Rouhan *et al.* (2004). These are indistinguishable morphologically except on the basis of perispore morphology: subsect. *Pachyglossa* has cristate, spinose, and perforate perispores, whereas those of subsect. *Platyglossa* are non-cristate, non-spinose, and non-perforate (Moran *et al.*, 2007).

The aim of the present study was to determine whether there was anything distinctive about the morphology of the gametophytes of *Elaphoglossum decursivum* that could potentially be useful in a phylogenetic context. Unlike nearly all previous studies of *Elaphoglossum* gametophytes, this one was carried out on gametophytes growing in nature, not in culture.

MATERIALS AND METHODS

This study was conducted during January 2013 at the Las Cruces Biological Station located near the town of San Vito, in Putarenas Province, Costa Rica (8°47'10"N, 82°57'40"W ca. 1200 m). Two populations of *E. decursivum* were sampled: one from the Wilson Botanical Garden and the other from a nearby secondary forest along the Río Java trail. The populations were photographed in the field and vouchers were made of the mature *E. decursivum* sporophytes (Matos *et al.* 2075, CR, NY, UPCB) and the host *Alsophila firma* (Matos *et al.* 2084, UPCB). Between 10–15 gametophytes were collected from each population. To reduce damage to the gametophytes during sampling, pieces of the underlying substrate surrounding one or more gametophytes were collected in the field. The gametophytes were then carefully separated from the *A. firma* root mantle in a laboratory setting and were washed in water when necessary. Many gametophytes had young sporophytes attached. All individuals were examined and measured using stereoscopes and light microscopes. Photographs were cropped and improved for colour balance using Adobe Photoshop © CS5 Extended, v. 12.0.4 ×64.

RESULTS

Gametophyte morphology.—The gametophytes of *Elaphoglossum decursivum* were most abundant on the trunks of *Alsophila firma* growing in partially shaded to relatively open areas (Fig. 1A–B). A few individuals were found on the trunks of angiosperms. Gametophytes were most frequently found 0.5–2 m from the ground, although it was not uncommon to see young sporophytes growing higher up the trunks. Gametophytes were strap-shaped, had crisped margins and terminated in an apical notch that measured 2–4 mm in width at the apex (Figs. 1D, 2A). Often the proximal part of the gametophytes had degraded leaving only the apical meristematic portion, giving the gametophyte a cordate appearance. A thickened midrib was absent, and none of the gametophytes branched. Reddish brown rhizoids extended from the ventral

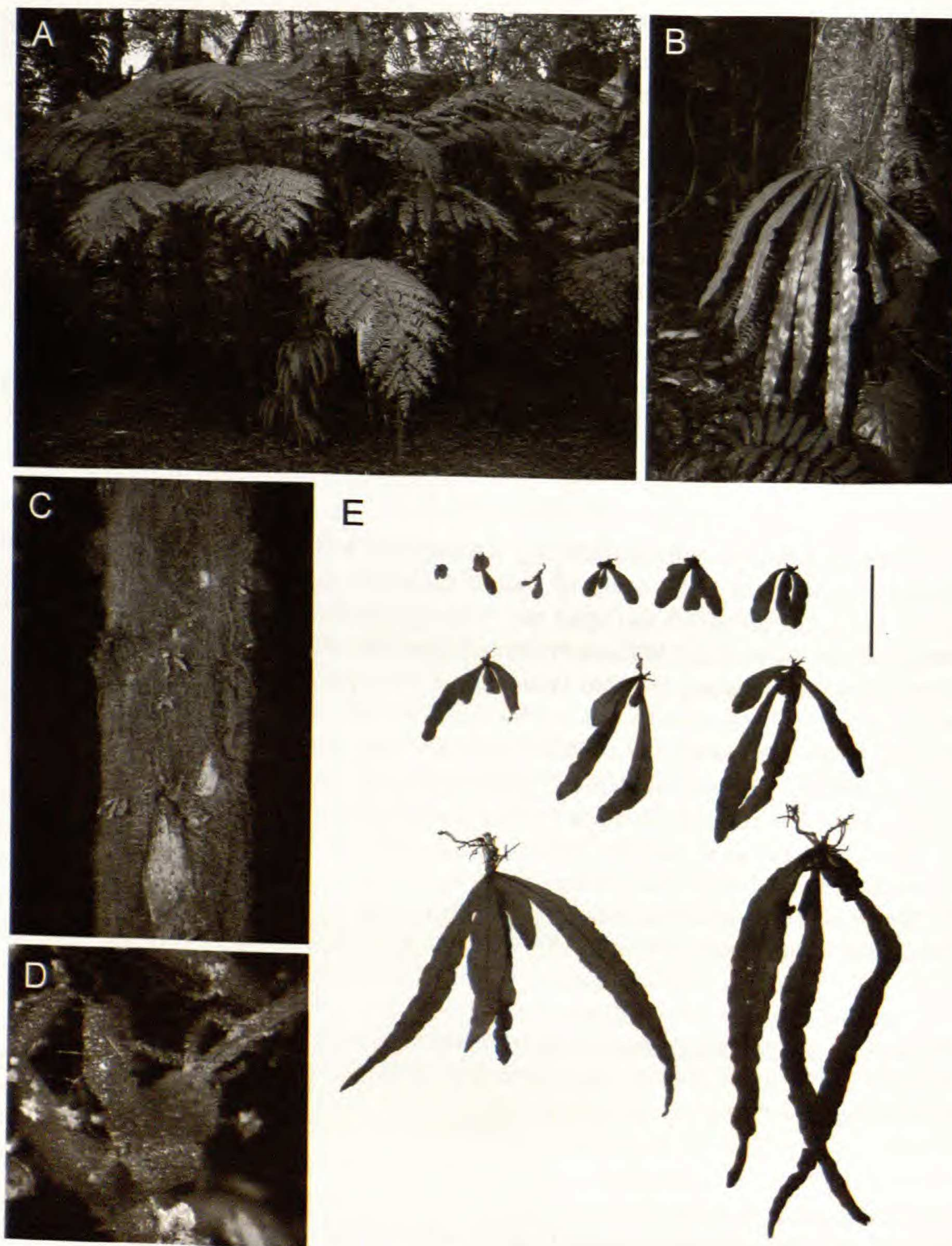


FIG. 1. A–E. Gametophytes, sporophytes, and habitat of *Elaphoglossum decursivum* at the Las Cruces Biological Station, Costa Rica. A. Habitat, showing large numbers of sporophytes growing on the lower trunks of *Alsophila firma*. B. Solitary sporophyte growing on an angiosperm. C. Gametophytes and young sporophytes on a trunk of *A. firma*. D. Mature gametophyte in the root [lower right]. E. Developmental series from a gametophyte [upper left] to a young sporophyte [lower right]; scale bar = 2.5 cm.

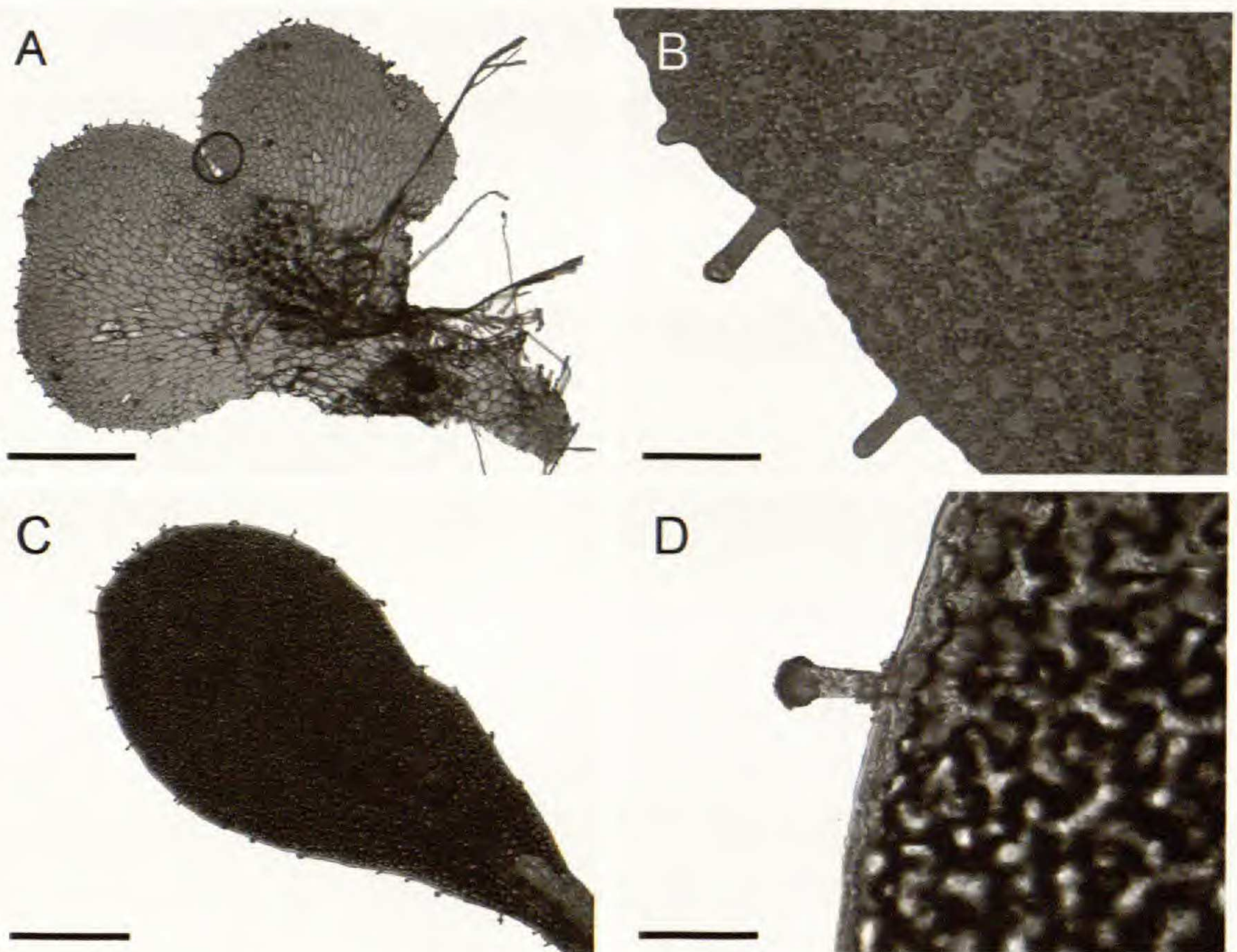


FIG. 2. Gametophyte (A–B) and first sporophyte leaf (C–D) of *Elaphoglossum decursivum*. A. Ventral view of a mature gametophyte, with a cluster of archegonia at the center, long castaneous rhizoids, and tiny unicellular marginal hairs. Black circle indicates apical notch. B. Margin of the gametophyte showing secretory, papillate-glandular hairs at different stages of development, from a tiny bulge on the surface of the cell to a fully extended secretory hair with waxy cap. C. First leaf of the sporophyte showing numerous unicellular hairs along its margin. D. Detail of a papillate-glandular hair with waxy cap on the margin of sporophyte lamina. Scale bar for A and C = 0.3 mm; scale bar for B and D = 75 μ m.

surface of the thallus. Unicellular glandular hairs, often with a whitish waxy tip, were found on the thallus surface and margins. These glandular hairs often contained chloroplasts, especially when young, and measured about 50 μ m long (Fig. 2B). We also observed minute, branched multicellular proscales (i.e., highly reduced, uniseriate scales; sensu Moran, 1986) on the surface of some of the gametophytes. These proscales often had swollen apical cells, as is typical of scales in many species of *Elaphoglossum* (Figs. 3A–B). Archegonia were located on the ventral surface near the apical notch (Fig. 3C). No antheridia were observed.

Young sporophyte morphology.—The surface and margins of the first few sporophyte leaves were covered with minute papillate-glandular hairs like those found on the gametophytes (Fig. 2C–D). With each successive new leaf, the glandular hairs decreased in abundance and became increasingly restricted to the margins. Glandular hairs were absent by time the young sporophyte produced leaves that were about 2 cm long. This corresponded to the fourth or

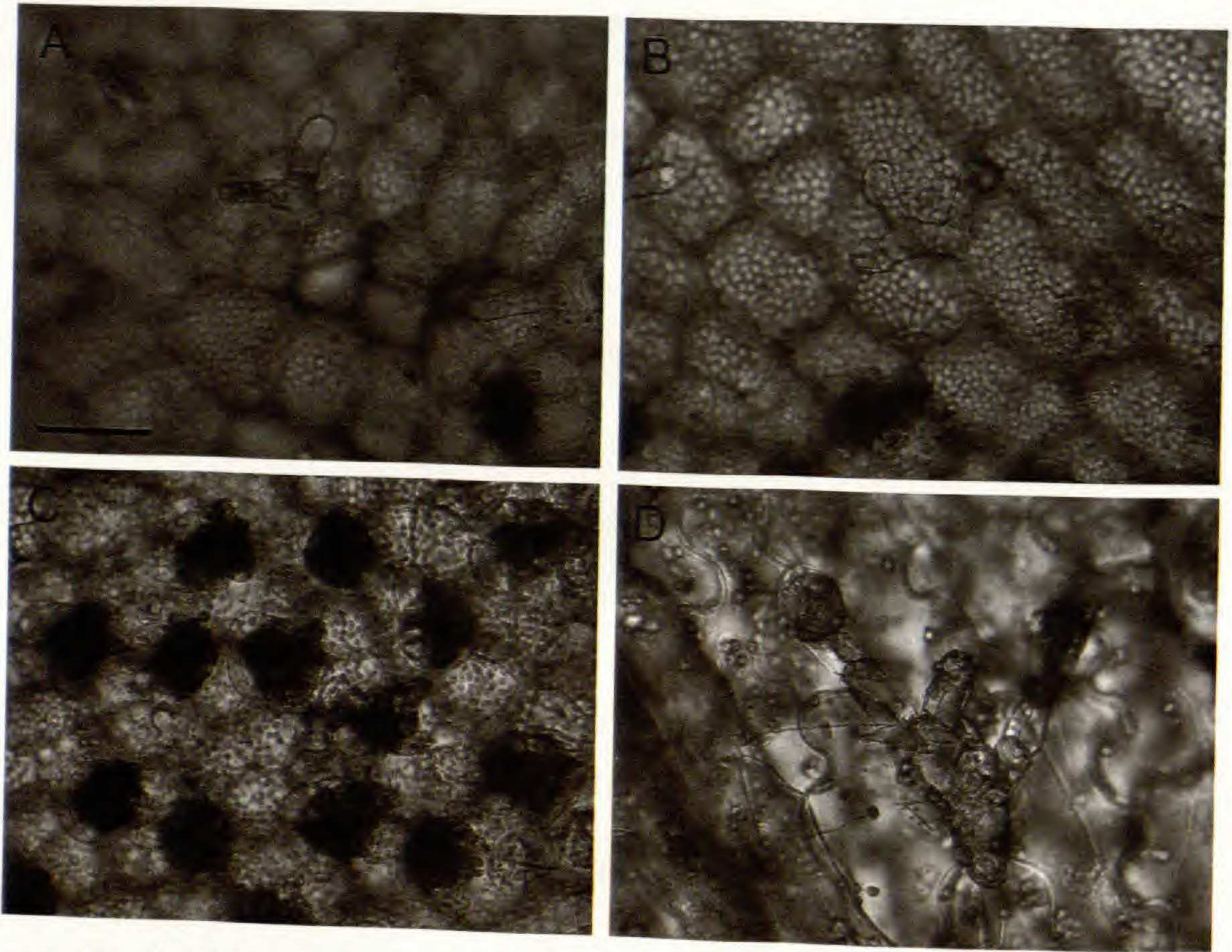


FIG. 3. Details of the gametophytes (A–C) and early sporophyte leaves (D) of *Elaphoglossum decursivum*. Scale bar = 75 μ m. A. Forked proscale on ventral surface of the gametophyte. B. Proscale on the ventral surface of the gametophyte. C. Dark neck cells of the archegonia, as seen from above. D. Proscale on the abaxial side of first sporophyte leaf. Note slightly swollen dark apical cells. Scale bar = 75 μ m.

fifth leaf (Fig. 1E). We also observed proscales on the abaxial surfaces of some of the young sporophyte leaf blades (Fig. 3D). These were difficult to observe because of their small size and were only seen on a few individuals.

DISCUSSION AND CONCLUSIONS

The gametophytes of *Elaphoglossum decursivum* share the same general morphology of most other species of the genus, the most prominent characters being the elongate, strap-shaped thallus and presence of papillate, glandular hairs on the margins and surface (Chiou *et al.*, 1998; Nayar and Kaur, 1971; Stokey and Atkinson, 1957). Unlike the gametophytes of many other *Elaphoglossum* species grown in culture, the field-collected gametophytes of *E. decursivum* lacked a prominent midrib, marginal rhizoids, and branched thalli. While there have been very few studies addressing the differences between field collected and culture-grown gametophytes, the work by Ranker and Houston (2002) and Skelton (2007) show that some differences are to be expected, especially with regard to the expression of sexual systems and the

dorsiventral presence of archegonia and rhizoids. The fact that several of the *E. decursivum* gametophytes were at least partially degraded seems to reflect the challenges of growing in a natural environment with limited resources. The continuous growth of these elongated thalli may also be an adaptation to growing in the dense root mantle of *Alsophila firma*, which is a highly heterogenous environment.

Chiou *et al.* (1998) found that gametophytes grown in culture from spores of five other *Elaphoglossum* species only produced archegonia after 8–24 months of growth. Taking this study as a guide, the presence of archegonia and the elongated thallus both suggest that the gametophytes observed were at least several months old, if not older. Perhaps spore germination and subsequent gametophyte growth started at the beginning of the wet season in early May. This observation is consistent with the idea that many fern epiphytes employ the strategy of having long-lived gametophytes (Chiou and Farrar, 1997; Farrar *et al.*, 2008; Watkins *et al.*, 2007). Chiou *et al.* (1998) did not find any evidence of an antheridiogen system in their study on *Elaphoglossum* gametophytes and hypothesized that intergametophytic mating was most likely for this group. As we did not find hermaphrodites or antheridia-producing gametophytes, our observations support this idea. However, locating the tiny, antheridia-producing gametophytes typical in a species with an antheridiogen system would be difficult in a field setting, so it is also possible that these individuals were present in our samples but were merely overlooked.

One interesting observation from this study was the finding of proscas (sensu Moran, 1986) on the surface of the *E. decursivum* gametophytes. As is typical of larger “normal” scales in most species of *Elaphoglossum* (Moran, personal observation), the apical cells of these proscas were slightly swollen with darkened contents. Most important, the proscas are exactly like those found on the leaves, where complete serial homology can be demonstrated between these minute proscas and larger “typical” scales. This finding of proscas is the first observation of scales on an *Elaphoglossum* gametophyte.

Without a comprehensive survey on the morphological characteristics of gametophytes from the various sections of *Elaphoglossum*, it is not possible to confirm the position of *E. decursivum* within sect. *Elaphoglossum* based on gametophyte morphology alone. Nevertheless, the characters observed in our study do not present any compelling evidence to the contrary. For instance, the presence of glandular hairs with waxy tips in *E. decursivum* is consistent with its position outside sects. *Squamipedia* and *Amygdalifolia*, as these two sections have been reported as having no hairs or hairs without waxy tips on their gametophytes, respectively. *Elaphoglossum decursivum* differs from sect. *Lepidoglossa* by lacking scales bearing many marginal teeth, each consisting of a single, acicular cell, which is diagnostic for sect. *Lepidoglossa* (Vasco *et al.*, 2009).

The two types of indument (i.e., papillate hairs and proscas) found on the very young leaves of *Elaphoglossum decursivum* represent the first observation of such indument in sect. *Elaphoglossum*. This observation raises questions on the evolution and development of glabrousness in this clade

and it would be interesting to see if the young sporophytes of other members of this clade have similar patterns of development.

Our study has shown that the gametophytes and young sporophytes of *Elaphoglossum decursivum* have additional morphological characters (i.e., papillate-glandular hairs) that are absent in the adult sporophytes. Studying these important stages in the fern life cycle not only provides a more complete picture of the biology of such organisms, but could also potentially be useful in a phylogenetic context, complementing the more traditional molecular and morphological studies.

ACKNOWLEDGMENTS

This study was conducted in January 2013 during the *Tropical Ferns and Lycophytes* specialty course sponsored by the Organization for Tropical Studies (OTS). The course was taught by Robbin Moran (The New York Botanical Garden) and James Eddie Watkins (Colgate University). We thank Miguel Cháves from OTS for his help with plant identification. We also thank two anonymous reviewers for their helpful comments and suggestions. This research was partially funded by grants to Matos by the Fulbright Scholarship Program and The CAPES Foundation, Ministry of Education of Brazil, to Choo by the Department of Plant Biology, Cornell University, and to Moran from the U.S. National Science Foundation (DEB1020443).

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